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Abstract

The elimination of fibroblast-like cells from primary cultures of fetal human livers was studied. A fibroblast-like cell line (HuF), which was obtained by subculturing fetal human liver cells 4 or more times, was briefly treated with hydrocortisone (HC) or putrescine (PUT). The growth of HuF cells was inhibited by HC at a concentration of 10(-2) M and by PUT at a concentration higher than 10(-3) M. Long-term treatment of HuF cells with 10(-3) M HC inhibited the growth of the cells. Primary cultures of fetal human livers were made in medium containing HC or PUT, and morphological and functional examinations were made. The cultures were predominantly composed of epithelial-like cells, with few fibroblast-like cells, when the HC concentration was 10(-5)M to 10(-3) M. A high amount of albumin was secreted at these concentrations of HC. On the other hand, at 10(-3) M PUT, many epithelial-like cells were seen, but albumin was undetectable. The present results indicate that albumin-producing epithelial-like cells can be selectively maintained in medium containing HC, in primary cultures of fetal human livers.

KEYWORDS: fibroblasts, human liver, hydrocortisone, putrescine

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An Attempt to Eliminate Fibroblast-Like Cells from Primary Cultures of Fetal Human Livers

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The elimination of fibroblast-like cells from primary cultures of fetal human livers was studied. A fibroblast-like cell line (HuF), which was obtained by subculturing fetal human liver cells 4 or more times, was briefly treated with hydrocortisone (HC) or putrescine (PUT). The growth of HuF cells was inhibited by HC at a concentration of 10^{-2}M and by PUT at a concentration higher than 10^{-3}M. Long-term treatment of HuF cells with 10^{-3}M HC inhibited the growth of the cells. Primary cultures of fetal human livers were made in medium containing HC or PUT, and morphological and functional examinations were made. The cultures were predominantly composed of epithelial-like cells, with few fibroblast-like cells, when the HC concentration was 10^{-5}M to 10^{-3}M. A high amount of albumin was secreted at these concentrations of HC. On the other hand, at 10^{-3}M PUT, many epithelial-like cells were seen, but albumin was undetectable. The present results indicate that albumin-producing epithelial-like cells can be selectively maintained in medium containing HC, in primary cultures of fetal human livers.

Key words : fibroblasts, human liver, hydrocortisone, putrescine

In the primary culture of normal human livers, hepatocytes are frequently replaced by an overgrowth of fibroblast-like cells during the early phase of the culture. Such growth of fibroblast-like cells often makes it difficult to study growth or differentiated functions of hepatocytes in culture.

Several attempts have been made to eliminate nonepithelial cells by culturing in medium including vitamine B complex, hexenolactone, steroide, or D-valine (1-4). The present study was aimed at eliminating the fibroblast-like cells from primary cultures of fetal human livers by the use of HC or PUT.

Materials and Methods

Specimens of fetal human livers were obtained at legal abortions. The fetal age ranged from the 16th to the 23rd week of gestation. Livers were minced and digested with 1000 u/ml dispase (Godo-shusei Co., Chiba, Japan) in Hanks' solution for 20 min at 37°C. The cell suspension was filtered and centrifuged 3 times at 50 g for 30 min. The pellet was resuspended in a growth medium and subjected to primary culture. The growth medium consisted of RPMI-1640 supplemented with 5 μg/ml insulin, 0.2% lactalbumin hybrolysate and 20% bovine serum. Primary cultures were made at 3×10^5 cells in 3 ml of the growth medium in 60-mm Falcon plastic dishes and grown in incubators in a 5% CO_2 atmosphere.

A fibroblast-like cell line (HuF), which was derived from passage cultures (4 or more times) of fetal human livers, was used in order to examine the effect of HC and PUT on the fibroblast-like cells. Multiwell plates (24 wells, Falcon) received 5×10^4 HuF cells in 0.5 ml of the growth medium containing HC (Sigma) or PUT (Sigma). Cell counting was made by a Coulter counter. For the detection of albumin in the media, spent media were collected from the monolayer cultures of fetal human livers cultured in medium containing HC or PUT for 19 days followed by culturing in HC- or PUT-free medium for 2 days. Detection of albumin was made by enzyme-linked immunosorbent assay (ELISA) using rabbit antihuman albumin and horse radish peroxidase conjugated rabbit anti-human albumin antibodies (Cappel, Cochranville, PA).

Results

As shown in Figs. 1 and 2, the growth of HuF cells was inhibited by treatment for 4 days with HC at a concentration of 10^{-2}M

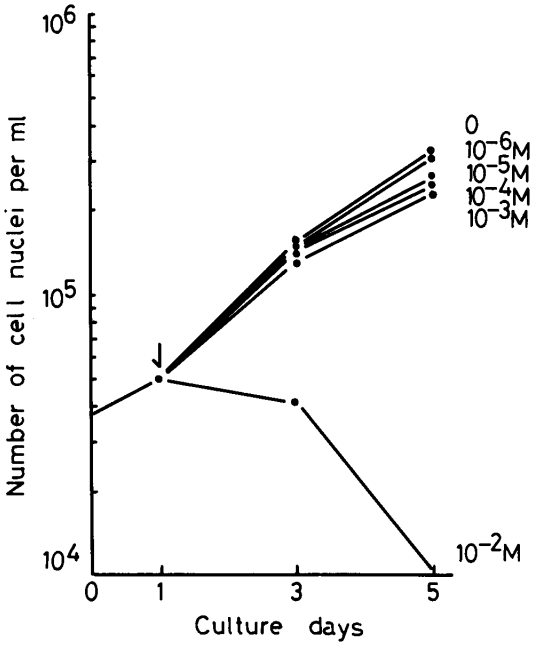

Fig. 1 Effect of hydrocortisone on the proliferation of fetal human liver fibroblasts (short-term experiments).

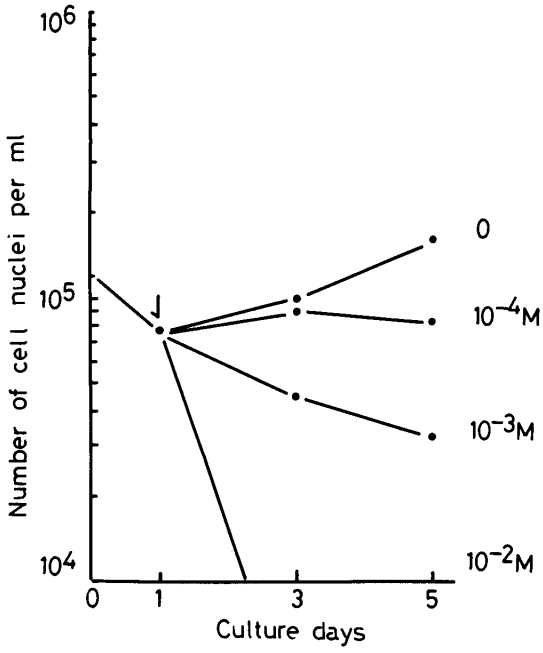

Fig. 2 Effect of putrescine on the proliferation of fetal human liver fibroblasts (short-term experiments).

and by PUT at a concentration of more than 10^{-3}M. HuF cells again revealed growth inhibition after long-term treatments over 9 days with HC at a concentration of 10^{-3}M (Fig. 3).

Primary cultures of fetal human livers were exposed to HC or PUT for 19 days, and morphological observation was made. Few fibroblast-like cells were seen when fetal human livers were cultured in the medium containing a high concentration of HC. On the other hand, there were a numder of fibroblast-like cells in the medium containing PUT, except at the highest concentration of PUT treated. In addition, epithelial-like cells had abundant cytoplasmic vacuoles in the PUT-treated cultures, suggesting degenerative alteration (Fig. 4).

Albumin in the culture media of primary cultures of fetal human livers, which were exposed to HC or PUT for 19 days, was examined ay ELISA (Table 1). In HC-media, epithelial-like cells retained the ability to

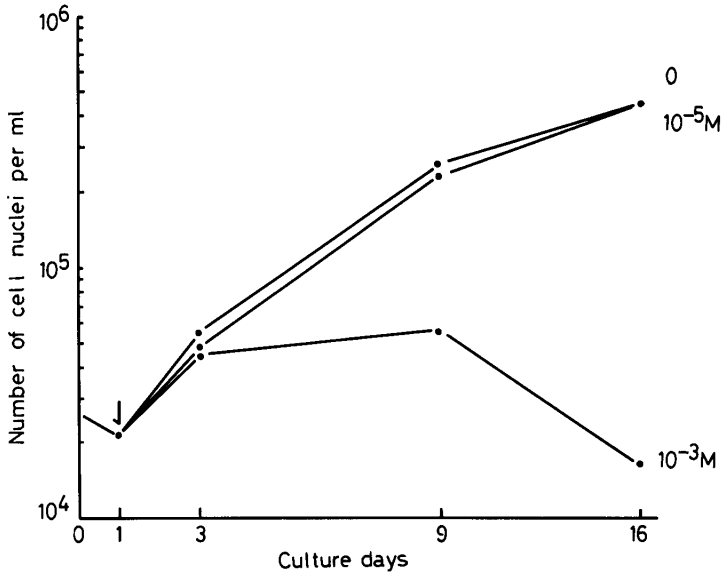

Fig. 3 Effect of hydrocortisone on the proliferation of fetal human liver fibroblasts (long-term experiments).

Table 1 Production of albumin in the media of primary cultures of fetal human livers treated with hydrocortisone or putrescine[a]

Treated with		Albumin (ng/ml)[b]
Non		20 ± 250
Hydrocortisone	10^{-3}M	620 ± 125
	10^{-5}M	120 ± 500
	10^{-7}M	100 ± 250
Putrescine	10^{-3}M	50 ± 25
	10^{-5}M	20 ± 15.6
	10^{-7}M	0

a : Media used for measurement were prepared as described in Materials and Methods.
b : Mean ± SD.

secrete high levels of albumin. On the other hand, in PUT, detectable amounts of albumin were not secreted.

Discussion

There are many papers concerning the effect of HC on the growth of fibroblast-like cells derived from human materials. HC stimulated the growth of human diploid fibroblasts, WI-38, and inhibited that of fibroblasts derived from human keratinocytes (5, 6). Whether the effect of HC is stimulatory or inhibitory may depend on cell types, the dose of HC, culture conditions and other factors. In the present study, HC showed an inhibitory action on the growth of a fibroblast-like cell line, HuF, by both short- and long-term treatments. Moreover, a number of fibroblast-like cells disappeared from primary cultures of fetal human livers in medium containing HC. Noyes reported that addition of HC to the medium resulted in a marked curtailment of fibroblastic growth when fetal human livers were subjected to explant cultures (1). Our study indicated that epithelial-like cells can be selectively maintained in medium containing HC in monolayer cultures of fetal human livers as well as in their explant cultures.

Few papers have been published in human tissue cultures by the use of PUT. In 1980, Stoner *et al.* reported that in medium containing 10 mM PUT, the outgrowth of human bronchial epithelial cells was similar to that observed in the control medium, but the outgrowth of bronchial fibroblasts was completely inhibited for periods of at least 4 weeks (8). In this study, there were few fibroblast-like cells and predominantly epi-

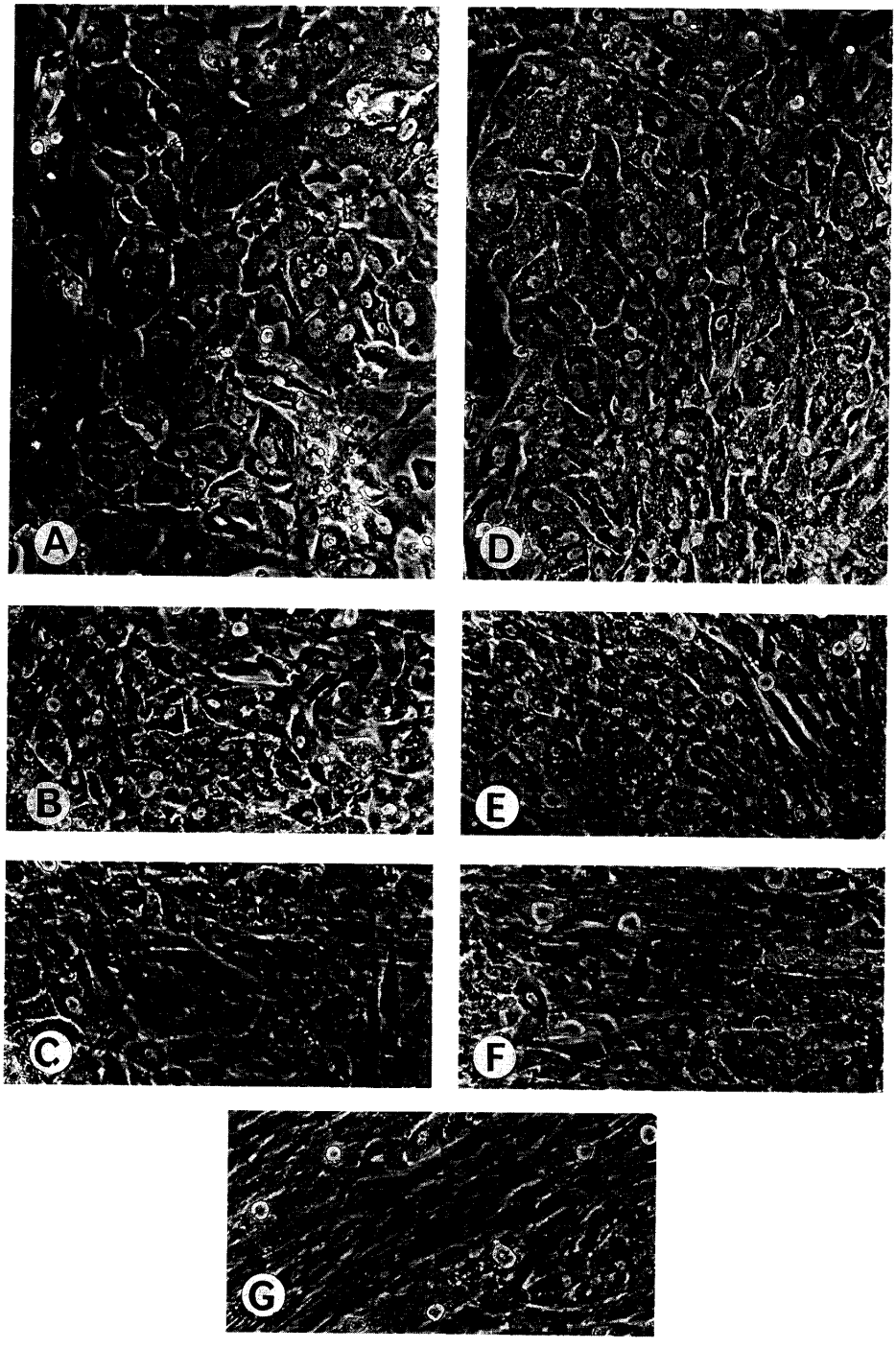

Fig. 4 Morphology of fetal human liver cells in primary culture treated with hydrocortisone or putrescine. A-C, hydrocortisone-treated (A, 10^{-3}M ; B, 10^{-5}M ; C, 10^{-7}M). D-F, putrescine-treated (D, 10^{-3}M ; E, 10^{-5}M ; F, 10^{-7}M). G, untreated control. Phase contrast. ×100.

thelial-like cells in the primary liver cell cultures treated with 10^{-3}M PUT, but the secretion of albumin was rarely observed. These findings indicate that the medium containing PUT does not provide conditions suited for the elimination of fibroblast-like cells from primary cultures of human livers.

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